REMARKS

Applicants appreciate the courtesy extended by Examiner Rao and his supervisor, Examiner Prouty, during an interview with Applicants' representative and one of the inventors, Dr. David Anderson, on September 16, 2003. During the interview, the Examiners suggested the revision of claims 1 and 32 to prescribe that "said enzyme cleavage interferes with host cell-pathogen binding in the intestines" and solicitation of publications evidencing that most enzymes do not survive entry into the intestines.

At the time of captioned Office Action, claims 1 and 21-72 were pending in the application. To expedite allowance, Applicants have amended claims 1 and 32, as recommended by the Examiners. The recitation of "in the intestines" is supported in the specification, for example, at page 4, lines 6-8; at page 8, lines 18-25; at page 24, lines 14-17 (Example II); and at page 26, lines 8-20 (Example III). In addition, Applicants have added claims 73-75, cancelled claims 37-38 and 65-68, and revised claims 39 and 40 to set forth the subject matter of the elected invention more clearly.

In light of *In re Ochiai*, Applicants request rejoinder of method-of-use claims, previously withdrawn, the language of which comports with revised composition claims 1 and 32. In anticipation of the allowance of the composition claims, Applicants have cancelled claims 21-31 and 33 and added method claims 76-94.

Upon entry of these amendments, claims 1, 32-36, 39-64, and 69-94 will be pending.

A detailed listing is presented, with an appropriate defined status identifier, of all claims that are or were in the application, irrespective of whether the claim(s) remain under examination. As noted, these amendments do not go beyond the original specification.

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and following remarks.

Claim Rejection Under 35 U.S.C. § 103

In the Official Action, the Examiner maintained the rejection of claims 1, 32, and 34-72 (except claims 56-65 and 69-72) over Beudeker, in view of Kuppe *et al.*, *J. Bacteriol.* **17**: 6077 (1989), and Barbis *et al.*, *Brazilian J. Med. Biol. Res.* **27**: 401 (1994). To overcome this rejection, Applicants have followed the Examiner's suggestions to amend composition claims 1 and 32 by inserting the phrase "in the intestines."

As to claim 32, moreover, Applicants' revisions incorporate some of the elements of cancelled claim 65, including the recitation that "said enzyme is a carbohydrase or a cerebrosidase." This qualification differentiates the claimed subject matter over the cited art, which teaches the use of phospholipase, itself an esterase and not a carbohydrase or a cerebrosidase.

Pursuant to another suggestion put forward by the Examiner at the September 16th interview, Applicants provide with this response copies of publications that evidence the instability of various enzymes in the gastrointestinal tract.

By approximating the conditions of the proventriculus of young chickens, for example, Kermanshahi and his colleagues completely inactivated a preparation of porcine lipase, which includes the lipase A2 used by Beudeker (EP 0743017A2). (The proventriculus is the low-pH digestive organ of the chicken, analogous to the stomach of a mammal.) The same conditions significantly reduces the activity of fungal and bacterial lipases. *See* Kermanshahi *et al., Poultry Sci.* 77: 1655 (1998) (Exhibit 1).

In light of the relevant background literature that Kermanshahi *et al.* illustrates, Applicants recall the point, emphasized at the September 16th interview, that none of Beudeker, Kuppe, and Barbis would have suggested the survivability of a phospholipase or a carbohydrase/cerebrosidase in the small intestine or lower gastrointestinal tract, thereby to bring about a therapeutic effect.* In sharp contrast,

* Of approximately 350 peptide and protein drugs under development for oral delivery, only two biotechnology protein drugs, whose chemical entity has been altered through the conjugation with ampiphilic oligomers (e.g., polyethylene glycol and alkyl groups), have been formulated successfully. *See* Soltero & Ekwuribe, *Innovation in Pharmaceutical Technology* (December, 2001), at pages 106-111 (Exhibit 2). Enteric or timed-release coatings and sophisticated protein modifications affecting bioavailability are too costly and impractical for the general use of animal feed. *Id.*

the presently claimed invention entails the use of an enzyme, as recited, that survives the harsh conditions of the gastrointestinal tract and yet reduces intestinal tract infections (see also the specification at page 11, lines 3-7). This aspect to the claimed invention, implicating oral administration for reduction of intestinal infections, would have been surprising to the skilled artisan and, hence, is supportive of non-obviousness. In other words, the person of ordinary skill would not have expected an enzyme, normally operating at neutral or alkaline pH, to survive in an acidic, protease-containing stomach and to reach the small intestine, there to prevent infections, as Applicants teach.

It is also unexpected that an enzyme as presently recited, exemplified by a bacterial toxin mixture of phospholipase C, has a curative effect (see specification at page 11, lines 26-28). The sources of the exemplified enzyme are from pathogens such as *Bacillus cereus* and *Staphylococcus aureus* (*id.*, at page 7, lines 22 and 27, and page 11, lines 22 and 23). Normally, toxins of any kind are carefully avoided in therapeutic preparations. In fact, PI-PLC injection in rabbits appears lethal and causes phosphasemia (*id.*, at page 11, lines 24-25). Thus, the skilled artisan surely would have had serious reservations to expend resources to test this invention with this information in mind.

In addition, an *in vitro* cell culture assay by Barbis cannot be predictive of an *in vivo* therapeutic efficacy of the tested enzyme (phospholipase C). *Cf.* Borchardt, *The Scientist* 15: 43 (2001) (Exhibit 3). Some of the key problems that show up after introduction into animal testing include toxicity, stability, and species-specificity. Applicants have mentioned already the potential toxicity and stability problems associated with enzymes as a class have been addressed in the above-mentioned sections. As for species-specificity, a drug candidate may work well in mice but not in humans. In view of these considerations, the skilled artisan certainly would not have generalized the results reported by Barbis, relating to canine parvovirus-infected feline cells *in vitro*, to an *in vivo* therapeutic efficacy.

Accordingly, there would have been no suggestion or motivation to employ Kuppe's and Barbis' phospholipase C as the "phospholipase" of Beudeker's feed composition, as exemplified by phospholipase A2. By the same token, the alleged *prima facie* case under Section 103, based on these references, cannot be sustained.

CONCLUSION

In view of the foregoing amendments and remarks, favorable reconsideration and allowance of this application are requested. An early notice in this regard is earnestly solicited. In the event that any issues remain, the Examiner is invited to contact the undersigned with any proposal to expedite prosecution.

Respectfully submitted,

Date 12 November 2003 By S. A. Bent

FOLEY & LARDNER
Customer Number: 22428
22428
PATENT TRADEMARK OFFICE
Telephone: (202) 672-5569
Facsimile: (202) 672-5399

Stephen A. Bent
Attorney for Applicants
Registration No. 29,768

Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

EXHIBIT 1

Stability of Porcine and Microbial Lipas s to Conditions that Approximate the Prov ntriculus of Young Birds

H. KERMANSHAHI, D. D. MAENZ, and H. L. CLASSEN[1]

Department of Animal and Poultry Science, Animal Science Building, 72 Campus Drive, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 5B5

ABSTRACT *In vitro* experiments were conducted to characterize the activity and the stability of lipase from animal (crude porcine, CPL; lyophilized porcine, LPL), fungal (*Rhizopus arrhizus*, RAL; *Aspergillus niger*, ANL), and bacterial (two *Pseudomonas* spp., PL1, PL2; and *Chromobacterium viscosum*, CVL) sources when exposed to conditions associated with the glandular stomach. Activity was measured at pH 3 to 8, 40 C and then monitored in response to temperature (40 C), time of exposure (0 and 30 min), pH (3 and 7), and pepsin level (5, 50, and 500 U/mL). All lipases except ANL and CVL had maximum activity at pH 7 to 8. The optimal pH for ANL and CVL were 5 and 6 to 8, respectively. Exposure of lipases to 40 C and pH 7 for 30 min reduced the activity of all lipases except ANL. In contrast, 40 C increased ANL activity 2.5-fold. Although activity of all lipases was reduced by exposure to pH 3, it was nearly eliminated for CPL and LPL. Pepsin concentration had only minor effects on lipase activity and then only at high concentration. The results demonstrate that bacterial lipases (PL1, PL2, and CVL) and ANL are more stable under conditions that approximate the glandular stomach and may explain why dietary porcine lipase has been ineffective in preventing fat malabsorption in previous *in vivo* studies.

(*Key words*: porcine lipases, microbial lipases, enzyme activity, pepsin, acidic pH)

1998 Poultry Science 77:1665–1670

INTRODUCTION

Studies on the digestibility of fats by chickens (Carew *et al.*, 1964) and turkeys (Sell *et al.*, 1986; Leeson and Atteh, 1995) have shown that the utilization of saturated fats is lower in young than in mature birds. The reasons for poor fat digestibility are not resolved but may reflect the underdeveloped state of gastrointestinal functions at the time of hatch. Potential reasons are a rapid feed transit time (Vergara *et al.*, 1989), and low levels of bile salt (Krogdahl and Sell, 1984) and pancreatic secretions (Noy and Sklan, 1995).

Relative to the adult, a lower digestibility of a given nutrient will occur in the young bird if the process of digestion and absorption is not complete during the period of gastrointestinal passage. Additives that are targeted toward improving the efficiency of the specific step or steps that limit the overall process of triglyceride digestion and absorption have the potential to increase fat digestibility in the young bird. In the young bird, bile salt secretion is lower and enterohepatic circulation of bile salts is higher than occurs in the adult (Jackson *et al.*, 1971; Green and Kellogg, 1987). However, dietary supplementation with a low amount of bile salts (0.025%) has resulted in only a small change in fat utilization (Gomez and Polin, 1976). In comparison with the digesta of humans and rats (Watkins, 1975), relatively high concentrations of bile salts (2 vs 14 m*M*/L) have been found in the 2-d-old chick (Green and Kellogg, 1987). These observations suggest that bile salt insufficiency may not be the primary cause of poor fat utilization in the young birds (Gomez and Polin, 1974, 1976).

Numerous reports indicate that the concentration of digestive enzymes in poultry increase with age (Krogdahl and Sell, 1989; Pubols, 1991; Noy and Sklan, 1995). Duodenal activity of lipase in young chicks increases 20 times between 4 and 21 d of age (Noy and Sklan, 1995). In turkeys, the lipase activities of pancreas and intestinal contents were relatively low in the newly hatched bird, increased slowly to about 6 wk of age, and then increased rapidly to 8 wk of age (Krogdahl and Sell, 1984, 1989). The relatively low rate of pancreatic lipase

Received for publication July 11, 1997.
Accepted for publication July 9, 1998.

[1]To whom correspondence should be addressed: Classen@admin.usask.ca

Abbreviation Key: ANL = *Aspergillus niger* lipase; CPL = crude porcine lipase; CVL = *Chromobacterium viscosum* lipase; K_m = Michaelis constant; LPL = lyophilized porcine lipase; NSP = nonstarch polysaccharide; PL1 and PL2 = two *Pseudomonas* spp. lipase sources; RAL = *Rhizopus arrhizus* lipase; TG = triglyceride; V_{max} = maximum velocity.

secretion in young birds may be insufficient for substantial triglyceride hydrolysis and therefore could contribute to poor fat utilization.

Dietary supplementation with lipase has the potential to improve fat utilization in situations of pancreatic lipase insufficiency. In humans, pancreatic enzyme replacement therapy is used routinely to treat malabsorption (Saunders and Wormsley, 1975). However, dietary supplementation with pancreatic lipase results in only a partial improvement in fat digestibility (DiMagno *et al.*, 1973). The use of dietary supplemented pancreatic lipase, with the exception of one paper (Polin *et al.*, 1980), has not been reported in poultry studies and most results are based on pancreatic insufficiency disease in humans (DiMagno *et al.*, 1977). Supplementation of crude porcine lipase (steapsin) in a corn-soybean diet containing 4% tallow was tested in young chicks but did not produce beneficial effects on fat utilization or bird performance (Polin *et al.*, 1980). Possible reasons as to why supplemental pancreatic lipase is ineffective or less effective than anticipated include destruction of pancreatic enzymes in the acid environment of the stomach, the variability in the potency of commercial pancreatic lipase supplements, and loss of pancreatic enzymes during passage through the small intestine (DiMagno *et al.*, 1977; Raimondo and DiMagno, 1994).

Dietary lipase can be protected from the adverse conditions of the gastric stomach by a number of methods. For example, gastric pH can be increased by the use of antacids or cimetidin, which inhibit gastric acid secretion. Lipase can be protected by the use of enteric coating procedures. Another possibility is to use enzyme sources that are stable under conditions of the stomach.

No information is available as to the relative stability of various native lipase sources under conditions of the avian proventriculus in young birds. The objective of this study was to evaluate the activity and stability of lipase sources after exposure to conditions that approximate passage through the proventriculus.

MATERIALS AND METHODS

A total of seven sources of lipase were used in this study. Crude porcine lipase (CPL, catalog no. L 3126), lyophilized porcine lipase (LPL, 50 kDa, catalog no. L 0382), and *Rhizopus arrhizus* lipase (RAL, 40 kDa, catalog no. L 4384), were obtained from Sigma,[2] *Aspergillus niger* lipase (ANL, 35 kDa, experimental), and *Pseudomonas* spp. lipase (PL1, 30 kDa, experimental) were obtained from Finnfeeds International[3] and *Chromobacterium viscosum* lipase (CVL, 73.5 kDa, catalog no. 5045), and *Pseudomonas* spp. lipase (PL2, catalog no. 6044) from Karlan Research Products Corp.[4]

Measurement of Lipase Activity

Lipase activity was measured in 10 mL of reaction mixture containing 1 m*M* tris-HCl buffer, 2 m*M* $CaCl_2$, 150 m*M* NaCl (Borgstrom and Erlanson-Albertson, 1973). The tributyrin (ICN)[5] concentration and the pH of the mixture were adjusted as required for the particular experiment. The reaction was initiated by addition of about 50 units of lipase activity and the rate of tributyrin hydrolysis monitored by continuously measuring the volume of a stock solution of NaOH added to the mixture to maintain pH over a 6-min period. Initial rates of tributyrin hydrolysis were calculated as the slope of the regression line for the linear portion of the reaction and expressed as micromoles fatty acids released per minute. Duodenal pH and the temperature of most birds are close to 7 and 40 C, respectively. These conditions, in the absence of any preincubation states, were chosen as the control. To determine the effect of temperature on lipase activity, the enzymes were exposed to 40 C for 30 min and then activity was measured at pH 7. Lipases were exposed to pH 3 for 30 min at 40 C and then activities were measured at pH 7 to see the effect of acidity on lipases. In order to study the effect of pepsin (catalog no. P 7125),[2] lipases were exposed to varying concentrations of pepsin for 30 min at pH 3, 40 C; activities were then measured at pH 7. Each assay was replicated three times. Protein concentration of lipases was measured spectrophotometrically at 595 nm using Sigma[3] procedure 610-A that employs Coomassie Brilliant Blue as a protein dye reagent.

Data were analyzed according to the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1985) as

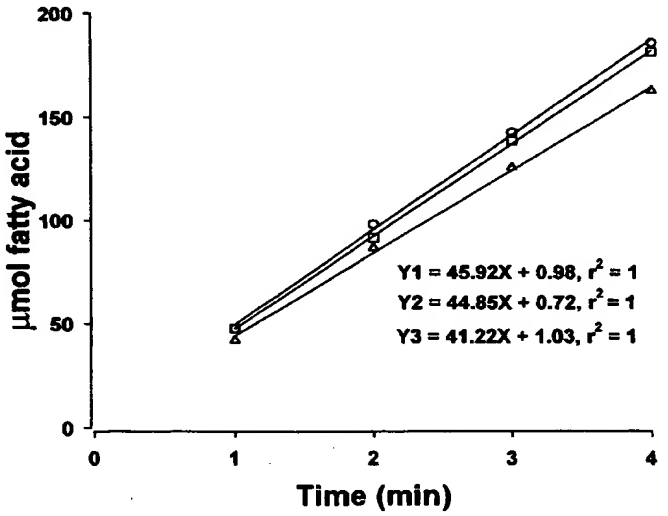

FIGURE 1. Activity of *Pseudomonas* spp. lipase (PL1) at 40 C, pH 7, using 2 mL tributyrin and ~50 unit lipase. Y1 to Y3 are the regression equations of replicates of each treatment. The slope of the equations represents the lipase activity.

[2]Sigma Chemical Co., St. Louis, MO 63178-9916.
[3]Finnfeeds International Inc., Marlborough, SN8 1AA, UK.
[4]Karlan Research Products Corp., Santa Rosa, CA 95403.
[5]ICN Pharmaceutical Inc., Costa Mesa, CA 92626.

a completely randomized design. When treatment effects were significant ($P < 0.05$), Duncan's multiple range test (Steel and Torrie, 1980) was used to compare means.

RESULTS

Initial Rate of Tributyrin Hydrolysis

Figure 1 shows the time course of 2 mL tributyrin hydrolysis at 40 C and pH 7. Under these conditions, the time course was linear for 4 min from initiation. In all subsequent experiments the initial rate of hydrolysis was defined as the slope of the linear portion of the time course of the reaction.

Kinetics of PL and CPL at pH 7 and 8

Figure 2 shows the effect of various concentrations of tributyrin on the initial rate of the reaction in the presence of PL1 and CPL at pH 7 and 8. Under these conditions the two lipases exhibited a Michaelis-Menten type of enzymatic activity. Increasing media pH from 7 to 8 had no effect on maximum velocity (V_{max}) but was associated with a slight decrease in the affinity of the substrate (K_m) for CPL. For both lipases near saturation of enzyme activity occurred at a substrate concentration of 2 mL. In all further experiments 2 ml of tributyrin was used to approximate the maximum activity of the lipases.

Effect of pH on Lipase Activity

Activity of lipases at 40 C and different pH in the absence of any pre-incubation period are presented in Figure 3. Enzyme activity tended to increase with higher pH. Little if any lipase activity was seen for all sources at pH 3 and 4. Maximum activity was seen in the neutral pH range (6 to 8) for all sources except ANL, which demonstrated maximum activity at pH 5. Unlike other sources with maximum activity at neutral pH, the activity of RAL was substantially lower at pH 8 than pH 7.

Effect of Preincubation Conditions on Lipase Activities

The results of preincubation of lipases under varying conditions are shown in Figure 4. Preincubation for 30 min at 40 C and pH 7, reduced the activity of all lipases except for ANL. Longer term exposure to 40 C resulted in a 2.5-fold increase in the activity of ANL measured at pH 7. Pre-incubation of lipases for 30 min at 40 C and in acidic

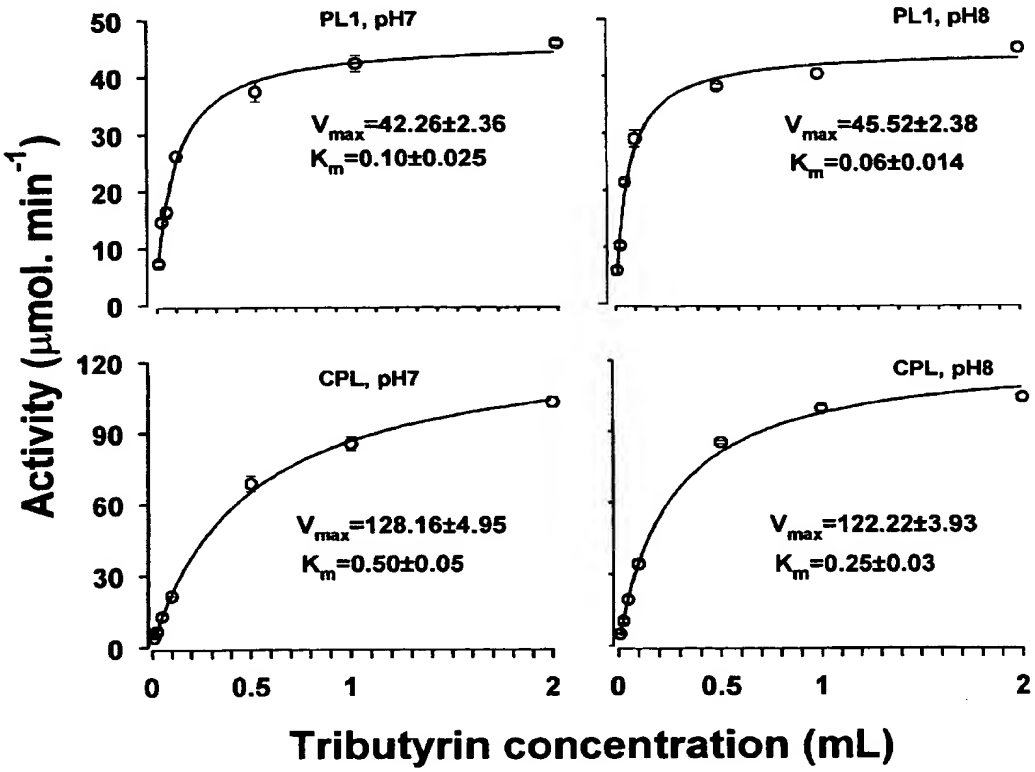

FIGURE 2. Activity of *Pseudomonas* spp. lipase (PL1) and crude porcine lipase (CPL) at pH 7 or pH 8 and different tributyrin concentrations. Assays were performed at 40 C, and ~50 units of lipase. V_{max} is the maximum velocity obtained under conditions of enzyme saturation and specified pH. K_m is the substrate concentration at which the enzyme has half-maximal velocity.

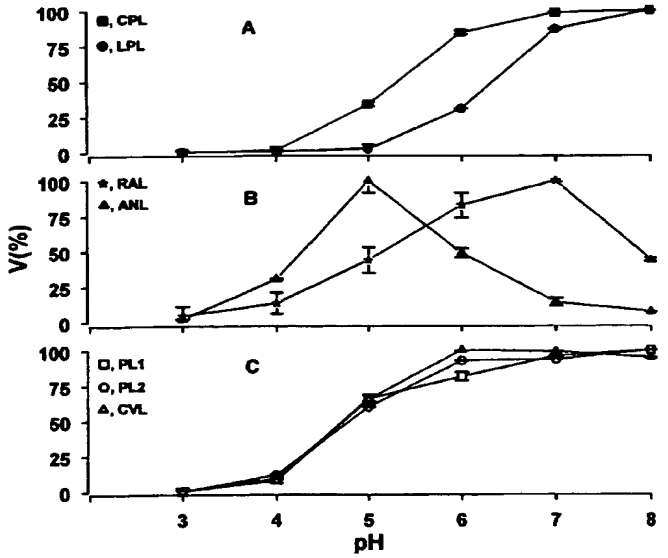

FIGURE 3. Activity of mammalian (A), fungal (B), and bacterial (C) lipases at varying pH, 40 C, and 2 mL tributyrin. V is the rate of hydrolysis in micromoles of fatty acid released per minute as a percentage of that obtained at maximum activity. The data points represent velocity (± SE) under the indicated conditions. CPL = crude porcine lipase; LPL = lyophilized porcine lipase; ANL = *Aspergillus niger* lipase; RAL = *Rhizopus arrhizus* lipase; PL1 and PL2 = *Pseudomonas* spp. lipase sources; CVL = *Chromobacterium viscosum* lipase.

condition (pH 3), markedly inhibited LPL and CPL activity, and reduced the activity of RAL, PL1, PL2, ANL, and CVL by 84, 66, 66, 64 and 51%, respectively ($P < 0.05$). Preincubation with 500 U/mL pepsin for 30 min at 40 C and pH 3, caused a slight but significant additional inhibition of CVL, PL1, and PL2 activities measured at pH 7. Preincubation of LPL, CPL, RAL, and ANL with pepsin had no effect on their activities.

DISCUSSION

The titrimetric methodology employed in this study provided an accurate measure of true initial rates of tributyrin hydrolysis. Thus, it was possible to test kinetic models for fit to initial rate data obtained with varying substrate concentrations. For each of the lipases tested, the data exhibited a Michaelis-Menten type of enzyme activity. Maximum velocities were obtained at substrate concentrations greater than 0.5 mL and thus in subsequent experiments initial rates of the reaction were measured with 2 mL of tributyrin incorporated in the mixture to insure that the reaction was measured at maximal velocity.

A measure of reaction rates at a high concentration of substrate provides a good indication of V_{max} that is independent of the physical degree of dispersion of the solution. Borgstrom and Erlanson-Albertson (1973) observed that when the substrate was dispersed by sonication, the apparent K_m was drastically decreased. They showed that an increasing amount of substrate concentration has the same effect as sonication of the substrate emulsion. Thus, using a high concentration of tributyrin allows the reaction to proceed at its maximal rate. This optimization assures a reliable titrimetric assay that follows the classical Michaelis-Menten kinetics.

The lipases tested varied in optimal pH, ranging from 5 to 8. Microbial lipases had higher activities at lower pH. The pH dependency for ANL obtained in our study is comparable with the results of Zentler-Munro *et al.* (1992), which demonstrated ANL activities in pH ranges of 2.5 to 5.5 with a pH optimum of 4.5. Our results with *Pseudomonas* lipase agree with Stead (1986), who reported that the pH and temperature optimums were in the range of 7 to 9 and 30 to 50 C, respectively.

Domestic chickens have an internal body temperature of approximately 40 C (Whittow, 1986), and feed passes through the low pH of the proventriculus (Winger *et al.*, 1962) in about 30 min (Noy and Sklan, 1995) before reaching the neutral pH of the small intestine (Duke, 1986). Exposure of most lipases to 40 C, for 30 min decreased their activities as expected; however, ANL showed an opposite response, increasing 2.5-fold in activity. This result was unexpected and requires further investigation.

Preincubation of CPL and LPL at pH 3 for 30 min (40 C) inhibited their activities when measured at pH 7. This result confirms the fact that porcine lipase is not acid resistant and inactivation of porcine or other mammalian pancreatic lipases before they reach the small intestine can contribute to the failure of oral enzyme replacement therapy for treatment of fat malabsorption (steatorrhea) in human studies (DiMagno *et al.*, 1977). Antacids, inhibitors of gastric acid secretion (Graham, 1982) or enteric coated lipase (Roberts, 1989) have been used to protect pancreatic lipase from acid denaturation. However, these methods are likely not applicable in the poultry industry because of the grinding activity of the gizzard and the lower retention time of feed in the duodenum. Enzyme replacement therapy with acid-stable lipases may be more appropriate in birds. Microbial lipases were more resistant to acidic conditions and it is probable that these enzymes should work better than pancreatic lipases.

Preincubation with pepsin under acid conditions that approximate the stomach is known to have variable effects on enzyme activity dependent upon the enzyme in question. Heizer *et al.* (1965) showed that trypsin was inactivated by pepsin and acid whereas pancreatic lipase was inactivated by a pH of less than 4 in human subjects. DiMagno *et al.* (1977) also showed that trypsin and pancreatic lipase can be inactivated by pepsin and the acid of the stomach, respectively. Zentler-Munro *et al.* (1992) showed that ANL is pepsin- and trypsin-resistant and any inhibition below pH 4 is completely reversible. In our study, incorporation of pepsin in the pH 3 preincubation media had no further inhibitory effect on the acid-sensitive lipases, CPL, LPL, and ANL.

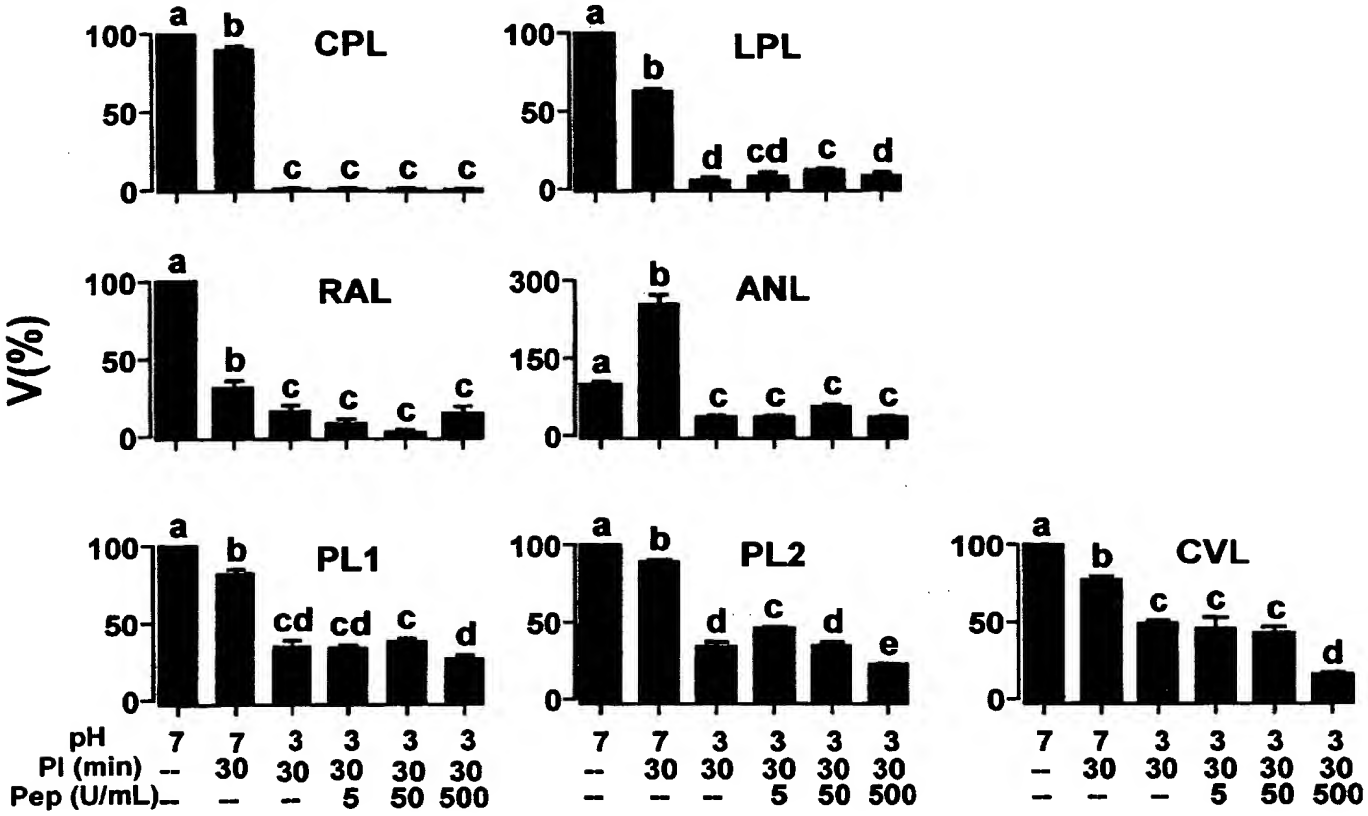

FIGURE 4. Activity of mammalian (top), fungal (middle), and bacterial (bottom) lipases under varying conditions at 40 C and using 2 mL tributyrin. V is the rate of tributyrin hydrolysis in micromoles fatty acid released per minute as a percentage of those obtained at maximum velocity. The data points represent velocity (± SE) under the indicated conditions. PI = preincubation time; Pep = pepsin level; CPL = crude porcine lipase; LPL = lyophilized porcine lipase; ANL = *Aspergillus niger* lipase; RAL = *Rhizopus arrhizus* lipase; PL1 and PL2 = *Pseudomonas* spp. lipase sources; CVL = *Chromobacterium viscosum* lipase. Bars for each lipase with different letters are significantly different ($P < 0.05$).

The activity of ANL and PL1 was not affected by pepsin addition to the acid preincubation media. At the highest concentration of pepsin the activity of PL2 and CVL was partially inhibited relative to the acid only preincubation treatment. Despite this loss, these sources still retained 16 to 27% of their maximal activity. Our findings are in agreement with Raimondo and DiMagno (1994) in that bacterial lipase lipolytic activity was shown to be more resistant to inactivation by acid than was the lipolytic activity of porcine lipase. As such, one would predict that bacterial lipases would retain more activity during passage to the small intestine than the lipolytic activity of either mammalian or fungal lipase.

In summary, not all lipases retain activity during incubation under conditions that mimic those of the chicken proventriculus. The activities of CPL, LPL, and RAL were very low after low pH exposure and therefore are not considered suitable candidates for use in the diet to prevent steatorrhea in young chickens. Despite some loss of activity, PL1, PL2, CVL, and ANL may be suitable sources of lipase for dietary supplementation. Further research is required to establish whether these enzymes can also be active in the conditions of the small intestine.

ACKNOWLEDGMENTS

The financial support of the Iranian Ministry of Culture and High Education and Finnfeeds International Inc., Marlborough, UK, is gratefully acknowledged.

REFERENCES

Borgstrom, B., and C. Erlanson-Albertson, 1973. Pancreatic lipase and colipase: Interaction and effect of bile salts and other detergents. Eur. J. Biochem. 37:60–68.

Carew, L. B., D. J. Hopkins, and M. C. Nesheim, 1964. Influence of amount and type of fat on metabolic efficiency of energy utilization by the chick. J. Nutr. 83: 300–306.

DiMagno, E. P., V.L.W. Go, and W.H.J. Summerskill, 1973. Relations between pancreatic enzyme outputs and malabsorption in severe pancreatic insufficiency. N. Engl. J. Med. 288:813–815.

DiMagno, E. P., J. R. Malagelada, V.L.W. Go, and C. G. Moertel, 1977. Fate of orally ingested enzymes in pan-

creatic insufficiency: comparison of two dosage schedules. N. Engl. J. Med. 296:1318–1322.

Duke, G. E., 1986. Alimentary canal: secretion and digestion, special digestive functions, and absorption. Pages 289–302 *in*: Avian Physiology. 4th ed. P. D. Sturkie ed. Springer-Verlag Inc., New York, NY.

Gomez, M. X., and D. Polin, 1974. Influence of cholic acid on the utilization of fats in the growing chicken. Poultry Sci. 53:773–781.

Gomez, M. X., and D. Polin, 1976. The use of bile salts to improve absorption of tallow in chicks one to three weeks of age. Poultry Sci. 55:2189–2195.

Graham, D. Y., 1982. Pancreatic enzyme replacement: the effect of antacids or cimetidine. Dig. Dis. Sci. 27:485–490.

Green, J., and T. F. Kellogg, 1987. Bile acid concentration in serum, bile, jejunal contents, and excreta of male broiler chicks during the first six weeks. Poultry Sci. 66:535–540.

Heizer, W. D., C. R. Cleavland, and F. L. Iber, 1965. Gastric inactivation of pancreatic supplements. Bull. Johns Hopkins Hosp. 116:261–270.

Jackson, B. T., R. A. Smallwood, and G. J. Piasecki, 1971. Fetal bile salt metabolism. 1. The metabolism of sodium cholate C^{14} in the fetal dog. J. Clin. Inves. 50:1286–1294.

Krogdahl, A., and J. L. Sell, 1984. Development of digestive enzymes and fat digestion in poults. Pages 352–354 *in*: 17th World's Poultry Congress, Helsinki, Finland.

Krogdahl, A., and J. L. Sell, 1989. Influence of age on lipase, amylase, and protease activities in pancreatic tissue and intestinal contents of young turkeys. Poultry Sci. 68: 1561–1568.

Leeson, S., and J. O. Atteh, 1995. Utilization of fats and fatty acids by turkey poults. Poultry Sci. 74:2003–2010.

Noy, I., and D. Sklan, 1995. Digestion and absorption in the young chick. Poultry Sci. 74:366–373.

Polin, D., T. L. Wing, P. Ki, and K. E. Pell, 1980. The effect of bile acids and lipase on absorption of tallow in young chicks. Poultry Sci. 59:2738–2743.

Pubols, M. H., 1991. Ratio of digestive enzymes in the chick pancreas. Poultry Sci. 70:337–342.

Raimondo, M., and E. P. DiMagno, 1994. Lipolytic activity of bacterial lipase survives better than that of porcine lipase in human gastric duodenal content. Gastroenterology 107: 231–235.

Roberts, I. M., 1989. Enzyme therapy for malabsorption in exocrine pancreatic insufficiency. Pancreas 4:496–503.

SAS Institute, 1985. SAS® User's Guide: Statistics. Version 5 Edition. SAS Institute Inc., Cary, NC.

Saunders, J.H.B., and K. G. Wormsley, 1975. Pancreatic extracts in the treatment of pancreatic exocrine insufficiency. Gut 16:157–162.

Sell, J. L., A. Krogdahl, and N. Hanyu, 1986. Influence of age on utilization of supplemental fats by young turkeys. Poultry Sci. 65:546–554.

Stead, D., 1986. Microbial lipases: their characteristics, role in food spoilage and industrial uses. J. Dairy Res. 53:481–505.

Steel, R.G.D., and J. H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw Hill Book Co., New York, NY.

Vergara, P., M. Jimenez, C. Ferrando, E. Fernandez, and E. Gonalons, 1989. Age influence on digestive transit time of particulate and soluble markers in broiler chicken. Poultry Sci. 68:185–189.

Watkins, J. B., 1975. Mechanisms of fat absorption and the development of gastrointestinal function. Pediatr. Clin. North Am. 22:721–730.

Whittow, G. C., 1986. Regulation of body temperature. Pages 221–224 *in*: Avian Physiology. 4th ed. P. D. Sturkie ed. Springer-Verlag Inc., New York, NY.

Winger, C. M., G. C. Ashton, and A. J. Cawley, 1962. Changes in gastrointestinal pH associated with fasting in laying hen. Poultry Sci. 41:1115–1120.

Zentler-Munro, P. L., B. A. Assoufi, and K. Balasubramanian, 1992. Therapeutic potential and clinical efficacy of acid-resistant fungal lipase in the treatment of pancreatic steatorrhea due to cystic fibrosis. Pancreas 7:311–319.

J Anim Sci. 2001 Jun;79(6):1621-30. Related Articles, Links

Resistance of feed enzymes to proteolytic inactivation by rumen microorganisms and gastrointestinal proteases.

Morgavi DP, Beauchemin KA, Nsereko VL, Rode LM, McAllister TA, Iwaasa AD, Wang Y, Yang WZ.

Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, AB, Canada, T1J 4B1. morgavid@em.agr.ca

Potential feed enzyme additives for ruminants were tested in vitro for their stability to ruminal microbial and gastrointestinal proteolysis. Four commercial preparations from Trichoderma longibrachiatum (A, B, C, and D) and one from an undisclosed source (E) were incubated up to 6 h with ruminal fluid taken from four lactating dairy cows before or 2 h after feeding. The stability of preparation B was also tested in the presence of pepsin at pH 3 and pancreatin at pH 7. Cellulase (EC 3.2.1.4), cellulose 1,4-beta-cellobiosidase (EC 3.2.1.91), beta-glucanase (EC 3.2.1.6), xylanase (EC 3.2.1.8), beta-glucosidase (EC 3.2.1.21), and beta-xylosidase (EC 3.2.1.37) activities were monitored throughout the incubations. Polysaccharidase activities of all enzyme preparations were remarkably stable in ruminal fluid taken after feeding. Ruminal fluid obtained before feeding inactivated the polysaccharidases in preparations B and D to a greater extent than ruminal fluid obtained after feeding. Cellulase and cellulose 1,4-beta-cellobiosidase activities were the least stable, declining ($P < 0.05$) by 35 and 60% for preparations B and D, respectively. Xylanase activity of preparation D decreased ($P < 0.05$) by up to 30% after 6 h of incubation, whereas beta-glucanase activity was not affected. The ability to degrade exogenous enzymes also differed among cows ($P < 0.05$). Pepsin and acid (pH 3.0) did not affect polysaccharidases in preparation B but decreased glycosidase activities by 10 to 15% ($P < 0.05$) after 1 h of incubation. Pancreatin, at the maximum concentration used, inactivated cellulase, cellulose 1,4-beta-cellobiosidase, and xylanase activities at a rate of 0.55, 1, and 0.45%/min, respectively. beta-Glucosidase and beta-xylosidase activities decreased by 1 and 0.75%/min, respectively. Partial proteolysis of cellulase, cellulose 1,4-beta-cellobiosidase, and xylanase by pancreatin produced a transient increase in activity. This twofold increase for cellulase and fourfold increase for cellulose 1,4-beta-cellobiosidase was directly proportional to pancreatin concentration. These results suggest that the enzyme feed additives tested were stable in the rumen of animals after feeding. Exogenous enzymes are likely to be more susceptible to the host gastrointestinal proteases in the abomasum and intestines than to ruminal proteases. However, exogenous polysaccharidases may survive for a considerable period of time in the small intestine and they probably maintain activity against target substrates in this environment.

EXHIBIT 2

The oral delivery of protein and peptide drugs

A variety of strategies can be used to overcome the barriers to protein/peptide drug delivery by the oral route.

Dr Richard Soltero and Dr Nnochiri Ekwuribe, Nobex Corporation

Currently, only two biotechnology drugs that can be given orally are known to be in clinical development in the US

The use of polypeptides and proteins for systemic treatment of certain diseases is now well accepted in medical practice. The role that these drugs play in replacement therapy is so important that many research activities are being directed towards the synthesis of large quantities by recombinant DNA technology. Many of these polypeptides are endogenous molecules that play an important role in maintaining organ function and metabolic homeostasis. Research institutions and pharmaceutical companies around the world have implemented strong research programmes to identify these proteins, their use and methods of producing them.

The availability of biotechnology products to develop is no longer an issue; there are over 350 peptide and protein drugs currently under development. However, delivering therapeutically active proteins and peptides by the oral route has been a challenge and a goal for many decades. Currently, only two biotechnology drugs that can be given orally are known to be in clinical development in the US. For such drugs to be absorbed through the intestinal tract, they must be protected from enzymes and must pass through the luminal barriers into the bloodstream. This article will present a review of strategies used to overcome the barriers to protein drug delivery by the oral route.

Routes of administration

The most common route of administration for proteins and peptides is by injection, although many other routes have been tried with varying degrees of success (1). Routes such as intranasal (2, 3), transdermal, buccal (4), intraocular and pulmonary will deliver the drug to the systemic circulatory system while avoiding transit through the digestive system. A major factor that limits the usefulness of these substances for their intended therapeutic application is that they are easily metabolised by plasma proteases when they reach the peripheral circulation. In addition, adverse effects associated with applying these drugs to the pulmonary or other mucosal surfaces may be limiting.

The oral route of administration for these substances is even more problematic because, in addition to proteolysis in the stomach, the high acidity of the stomach destroys them before they reach the intestine for absorption. Polypeptides and protein fragments, produced by the action of gastric and pancreatic enzymes, are further cleaved by exo- and endopeptidases in the intestinal brush border membrane to yield di- and tripeptides, and even if proteolysis by pancreatic enzymes is avoided, polypeptides are subject to degradation by brush border peptidases. Any of the peptides that survive passage through the stomach are further subjected

to metabolism in the intestinal mucosa, where a penetration barrier prevents entry into cells. On the other hand, oral and colonic delivery have the advantage of delivering drugs through the intestinal tract to the hepatic portal vein, and then to the systemic circulation. The ease of administration and higher degree of patient compliance with oral dosage forms are the major reasons for preferring to deliver proteins and peptides by mouth.

In spite of the obstacles to gastrointestinal survival, there is substantial evidence in the literature to suggest that nutritional and pharmaceutical proteins are absorbed through the intestinal mucosa, although in minute amounts (5). Small amounts of peptide drugs can be absorbed by the action of specific peptide transporters in the intestinal mucosa cells; this suggests that properly formulated protein or peptide drugs may be administered by the oral route with retention of sufficient biological activity for their therapeutic use.

Strategies for oral delivery

Formulating for delivery through the gastrointestinal (GI) tract requires a multitude of strategies. The dosage form must initially stabilise the drug, while making it easy to take orally. Once delivered to the stomach or intestinal tract, the protein has to be protected from enzymatic degradation since digestive processes are designed to break down proteins and peptides without any discrimination in favour of therapeutically active compounds.

One strategy for overcoming the body's natural processes is to alter the environment for maximum solubility and enzyme stability of the protein by using formulation excipients such as buffers, surfactants and protease inhibitors. If the enzyme attack can be defeated or delayed, the proteins can be presented for absorption. Earlier work by Abuchowski and Davis (6) showed that proteins and peptides could be derivatised with polyethylene glycol (PEG) to achieve properties such as retention of activity, prevention of immunogenicity and prevention of excessive enzymatic degradation. More recently, Nobex has introduced the use of oligomers with both hydrophilic and lipophilic properties (7); these oligomers confer the enzymatic stability necessary for proteins to survive the digestive processes in the gut.

If a protein drug has the stability and enzyme resistance necessary to survive transit through the stomach and into the intestine, it is then faced with a number of lipophilic and hydrophilic barriers to cross (Figure 1). The drug must first dissolve in the contents of the intestinal lumen if it is not already in solution; then there is a mucus layer and a water layer protecting the surface of the epithelial cells. The protein or peptide drug must have sufficient water - and lipid-solubility to pass through these layers. The epithelial tissue represents the next barrier. There are cases where a protein can be absorbed into these cells by endocytosis, and then transported to the basement membrane on its way to the capillaries.

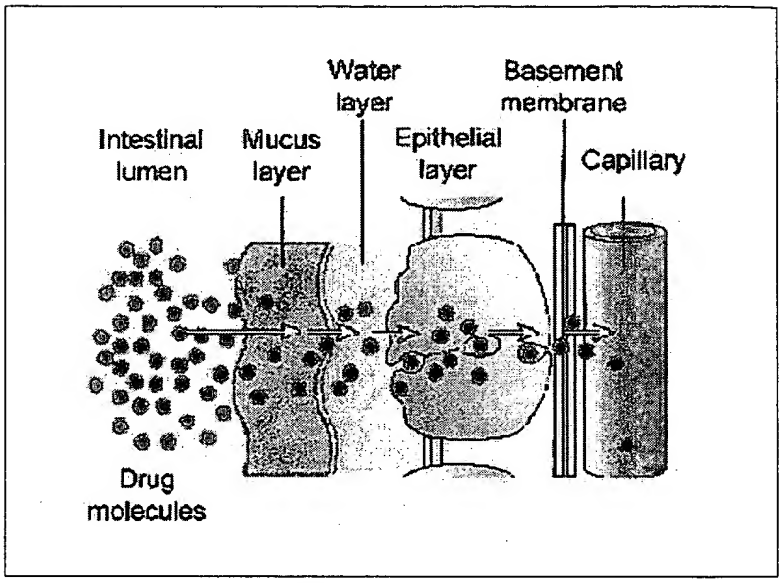

Figure 1. Barriers to the absorption of a drug in the intestine.

Another strategy for oral delivery, therefore, is to promote absorption through the intestinal epithelium. Absorption may be enhanced when the product is formulated with acceptable safe excipients (8). A typical transport mechanism for proteins across the epithelial boundary is paracellular transport. There are tight junctions between each of the cells in the epithelium that prevent water and aqueous soluble compounds from moving past these cells. A number of absorption enhancers are available that will cause these tight junctions to open transiently, allowing water-soluble proteins to pass. Fatty acids, surface-active agents, EDTA, glycerides and bile salts have all been shown to be effective in opening these tight junctions.

Most of the data showing the successful oral delivery of model proteins, such as insulin and calcitonin, has been generated in animal studies. These compounds have been combined with a variety of excipients and formulated in unique delivery systems to attain measurable systemic levels after oral delivery. In many cases, the formulation approaches would not be appropriate for human delivery; however, one can learn much from these model systems. For example, adding sodium cholate (9) or soybean trypsin inhibitor (10) was effective in delivering insulin to mice and dogs. Fatty acids also help, and are thought to

...there is substantial evidence in the literature to suggest that nutritional and pharmaceutical proteins are absorbed through the intestinal mucosa, although in minute amounts

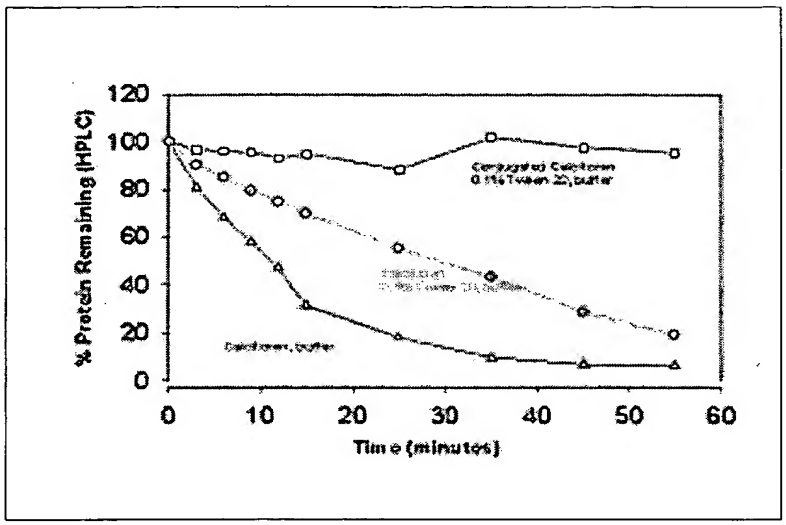

Figure 2. Enzyme stability of calcitonin incubated in a buffer with chymotrypsin at 37°C. Adding a surfactant to the buffer protects the compound and enzyme stability is improved. An ACE form of conjugated calcitonin in the same medium becomes inherently stable, and shows no loss of protein even after one hour.

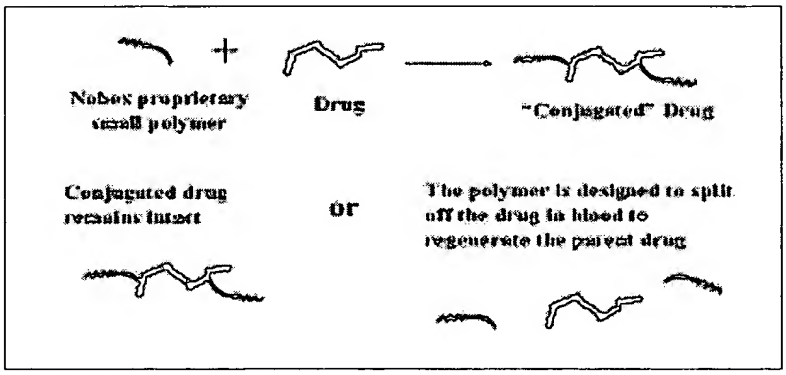

Figure 3. Nobex conjugation technology.

A compromise may lie in the balance of hydrophobic and hydrophilic characteristics of the oligomer used in the modification of protein drugs ...

interact with the lipid layer of epithelial cells causing a temporary disruption that can allow certain proteins to be absorbed in animals (11).

One more way to enhance absorption is to deliver the protein to the lower GI tract where absorption of proteins is more easily facilitated. To achieve this end, the formulator can apply standard drug delivery techniques, such as enteric coatings or sustained release.

Altered chemical entities

Drug molecules or their formulations must facilitate both aqueous and lipid layer penetration for proteins to be absorbed. Results indicate that simply increasing lipophilicity is not sufficient to improve transcellular or paracellular transport to the point where therapeutic levels of a drug can be absorbed. On the other hand, increasing the hydrophilicity of protein drugs does not increase paracellular transport, nor does it facilitate transcellular transport. A compromise may lie in the balance of hydrophobic and hydrophilic characteristics of the oligomer used in the modification of protein drugs, resulting in an altered chemical entity (ACE). This technique has worked successfully for several proteins at Nobex.

The Nobex technology involves the bonding of polyethylene glycol (PEG) and alkyl groups or fatty acid radicals to produce desired amphiphilic oligomers. These oligomers are conjugated to proteins or peptides to obtain desired amphiphilic products that can traverse the aqueous and lipid layers of the mucosa, and can resist excessive degradation of protein or peptide drugs. In the case of protein drugs, the attachment of the oligomers prevents the product from existing in multiple self-association forms; reduced self-association ACEs enable the product to penetrate through the epithelial walls. Nobex oligomers, being amphiphilic, enable the protein drugs to maintain close interaction with the mucosal wall, and are more compatible with formulation excipients than non-conjugated proteins.

An example of how this technology improves enzyme stability is shown in Figure 2. The enzyme stability of calcitonin when incubated in a buffer with chymotrypsin at 37°C is poor. Adding a surfactant to the buffer protects the compound and enzyme stability is improved. An ACE form of conjugated calcitonin in the same medium becomes inherently stable, and shows no loss of protein even after one hour. The activity of the compound was not affected by this conjugation.

Nobex has created a series of modified proteins that provide high water- and lipid-solubility and are resistant to enzyme attack. The technology consists of conjugating one or more oligomers to selected sites on the protein or peptide. As indicated in Figure 3, these conjugations can be either covalently bound to form an ACE conjugated drug, or loosely bound so they hydrolyse off in the plasma to regenerate the parent drug.

This conjugation technology has been applied to several proteins, including insulin. Historically, insulin has been a model compound for oral delivery techniques, and many investigators have tried and failed to deliver the native compound using formulation and drug delivery techniques. Nobex's conjugated insulin consists of a short chain PEG linked to an alkyl group which, in turn, is linked to LYS-29 of the beta chain. Biological activity is retained and the compound is readily absorbed from the GI tract.

Oral delivery of insulin

This conjugated protein is known as hexyl insulin monoconjugate 2 (HIM2), and is the first successfully delivered oral dosage form of insulin to show good oral bioefficacy in humans. This ACE has been studied in Phase I and II clinical trials in Europe, and in five Phase II clinical trials in the US. The pharmacokinetic profile mimics insulin secreted from the pancreas, since the drug is delivered through the GI tract and through the hepatic portal vein. The oral form of insulin goes directly to the liver and is believed to stimulate normal biochemical pathways, including glucose control.

HIM2 was given to fasted Type I diabetic patients in two sequential doses as shown in Figure 4. Type I patients are distinguished from Type II patients in that Type I have no ability to produce their own endogenous insulin, whereas Type II have some insulin production. In the absence of insulin control, a Type I diabetic's blood glucose level will increase to a point where the patient becomes ketoacidotic with serious and even fatal consequences. When HIM2 was administered to fasted patients, their glucose levels stabilised and remained at normal levels for over four hours. Additional clinical trials in both Type I and Type II populations have substantiated these findings.

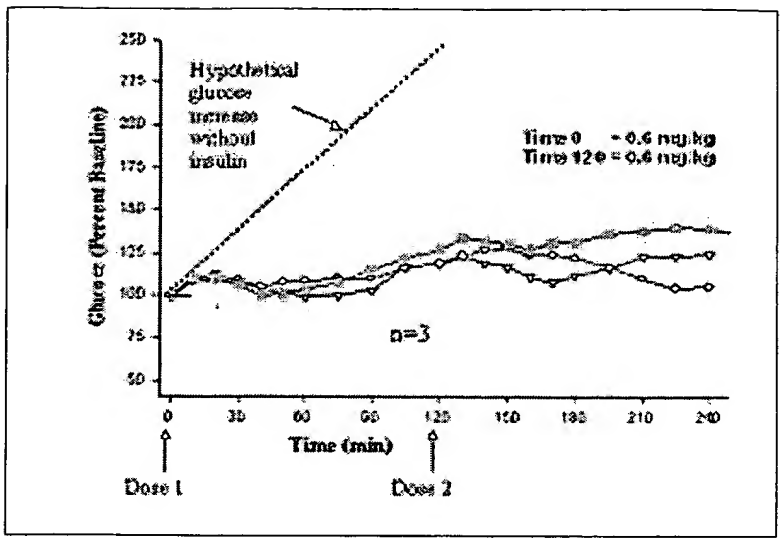

Figure 4. Fasted Type I diabetics' glucose response to sequential oral doses of conjugated insulin.

Human clinical results with conjugated insulin are a clear demonstration that a protein can be developed into a therapeutically viable product. With the conjugated form of insulin and an appropriate formulation, a product can be developed which has the attributes required for oral delivery. It survives the enzyme-rich environment of the intestinal tract, and crosses the various boundaries of the lumen to be absorbed into the bloodstream. Once into the circulation, it demonstrates the desired effect of controlling blood glucose

Conclusion

Human clinical results with conjugated insulin are a clear demonstration that a protein can be developed into a therapeutically viable product

Oral delivery of peptides and proteins for the therapeutic treatment of disease is now possible. These drugs can make the passage through the intestinal tract, through the luminal barriers and into the bloodstream. Strategies used to overcome these barriers include employing proper excipients for enzyme resistance and absorption enhancement. Common drug delivery techniques - such as sustained release and targeted delivery - may also assist. The most successful technique is the altered chemical entity, which is designed to work in combination with formulation strategies to achieve a protein product that is orally available in humans.

Dr Richard A Soltero is currently Executive Director of Pharmaceutical Development at Nobex Corporation. Prior to this position, he spent 25 years in Pharmaceutical R&D at Ciba-Geigy, Berlex and J&J, and five years in contract research organisations. Previous positions included Vice President of Laboratory Operations at Magellan Laboratories and at AAI, and Head of Worldwide Analytical, Microbiology and Stability R&D at SmithKline Beecham Consumer Healthcare.

Dr Nnochiri Ekwuribe received a PhD degree in organic medicinal chemistry from the University of California, San Francisco. His most recent work has been in the area of drug delivery systems and particularly in the oral delivery of protein/peptide drugs, and he is the inventor of the amphiphilic polymer technology licensed to Nobex. He is currently Vice President of Chemistry Innovations and Drug Discovery at Nobex.

References

1. Wearley LL (1991). Recent progress in protein and peptide delivery by noninvasive routes. Crit Rev Ther Drug Carrier Syst, 8, 4, 331-94.
2. Torres-Lugo M and Peppas NA (2000). Transmucosal delivery systems for calcitonin: a review. Biomaterials 21, 12, 1191-6.
3. O'Hagan DT and Illum L (1990). Absorption of peptides and proteins from the respiratory tract and the potential for development of locally administered vaccine. Crit Rev Ther Drug Carrier Syst 7, 1, 35-97.
4. Sayani AP and Chien YW (1996). Systemic delivery of peptides and proteins across absorptive mucosae. Crit Rev Ther Drug Carrier Syst, 13, 1-2, 85-184.
5. Lee YH and Sinko PJ (2000). Oral delivery of salmon calcitonin. Adv Drug Deliv Rev, 42, 3, 225-38.
6. Abuchowski A and Davis FF (1979). Preparation and properties of polyethylene glycol-trypsin adducts. Biochim Biophys Acta, 578, 1, 41-6.
7. Radhakrishnan B, Rajagopalan J, Anderson WL, *et al.* (1998). Structure-activity relationship of insulin modified with amphiphilic polymers. Pharm Sci 1, 1, S-59. (American Association of Pharmaceutical Scientists, San Francisco, CA.)
8. Sinko PJ, Lee YH, Makhey V *et al.* (1999). "Biopharmaceutical approaches for developing and assessing oral peptide delivery strategies and systems: *in vitro* permeability and *in vivo* oral absorption of salmon calcitonin (sCT). Pharm Res, 16, 4, 527-33.
9. Hosny EA, Ghilzai NM, *et al.* (1997). Promotion of oral insulin absorption in diabetic rabbits using pH-dependent coated capsules containing sodium cholate. Pharm Acta Helv, 72, 4, 203-7.
10. Ziv E, Kidron M, *et al.* (1994). Oral administration of insulin in solid form to nondiabetic and diabetic dogs. J Pharm Sci, 83, 6, 792-4.
11. Muranishi S (1997). Delivery system design for improvement of intestinal absorption of peptide drugs. Yakuga.

EXHIBIT 3

The Scientist 15[5]:43, Mar. 5, 2001

OPINION

Integrating Drug Discovery and Development

By Ronald T. Borchardt

Illustration: Anthony Canamucio

In response to increased economic pressures, research-based pharmaceutical companies are attempting to streamline and accelerate their drug discovery and development processes. With the advent of genomics, proteomics, and bioinformatics, these companies now have access to an unprecedented abundance of potential therapeutic targets. With the emergence of combinatorial chemistry and innovative robotic-based technologies for conducting high throughput screening, these companies can also rapidly identify chemical leads that interact with these potential therapeutic targets. Using rational drug design strategies, promising leads can be rapidly optimized into high affinity ligands, referred to in the industry as new chemical entities (NCEs).

While these new technologies have enhanced the output of drug discovery groups, the quality of NCEs, as measured by their success in preclinical and clinical development, has not improved significantly. For example, less than 40 percent of NCEs produced by drug discovery groups survive preclinical testing and eventually undergo human clinical testing. Of these, less than 10 percent ultimately come through clinical trials and become marketed products. This greater-than-90 percent attrition rate is a serious problem that could jeopardize a company's long-term growth and its commercial viability.

In the mid 1990s, research-based pharmaceutical companies began asking themselves, "Why do NCEs fail in preclinical and clinical development?" The primary reasons for failure were lack of efficacy, toxicity, and poor biopharmaceutical properties. Roughly 40 percent of the failures in preclinical and clinical development were attributable to poor pharmaceutical properties, including chemical instability, low aqueous solubility, poor cell permeation characteristics, and high rates of metabolism and/or clearance by the liver or kidney.

In response, many companies began to develop strategies and technologies that could be used early in drug discovery to assess the "drug-like characteristics" of chemical leads that have shown activity in high throughput screens. These strategies and technologies were also used in the optimization of chemical leads into NCEs. The hope was that NCEs discovered in this way would have better "drug-like characteristics," increasing their probability of success in preclinical and clinical development. Many companies established "developability" criteria based on these "drug-like characteristics". The "developability" criteria, in addition to standards for receptor-based potency and specificity, became important considerations in selecting NCEs for preclinical and clinical development.

ADME characteristics (A, absorption; D, distribution; M, metabolism; E, elimination) are included in these "developability" criteria. The ADME characteristics are particularly important in determining whether or not a molecule can be developed in an oral dosage form. To illustrate how companies have tried to integrate what were traditionally drug development activities into drug discovery, I discuss below the use of ADME characteristics to predict the oral bioavailability of an NCE.

In the past, medicinal chemists and pharmacologists in drug discovery groups have used the term "oral bioavailability" to indicate that an NCE has pharmacological activity when administered orally. The production of a marketable dosage

form (e.g., tablet, capsule, or solution) from this promising NCE then became the responsibility of the development scientist. The development scientist, however, understood "oral bioavailability" to mean the fraction of an oral dose reaching the systemic circulation, and quantified bioavailability using measured concentrations of the drug in the bloodstream. Unfortunately, the "activity-based bioavailability" of the medicinal chemist and pharmacologist often did not correlate with the "blood-level based bioavailability" of the development scientist. This lack of agreement can occur because the blood level of a drug may not be correlated with its activity, or may be correlated in a highly nonlinear manner.

As the pharmaceutical industry began to assess "drug-like characteristics" early in drug discovery, further problems arose between discovery and development scientists. First, it became readily apparent that the determination of oral bioavailabilities (i.e., by measuring blood levels) was not feasible at the stages of lead selection and lead optimization, due to the large number of compounds involved. As a result, most companies have taken a reductionist approach to the problem, using high throughput screens (HTS) to rapidly assess those individual factors (e.g., solubility in the lumen of the intestine, chemical stability in the stomach and the small intestine) that could ultimately influence the blood levels of a compound after oral dosing.

While these assays provide useful information about the "drug-like characteristics" of a molecule, none can be used in isolation to predict oral bioavailability unless the critical factor(s) that limit oral bioavailability (e.g., low solubility, low mucosal permeation, and high metabolism) has been clearly identified for that compound. The interpretation of isolated data by scientists from different disciplines can be very misleading. For example, as a consultant with pharmaceutical companies, I often hear medicinal chemists claim, "My compound shows good permeation across Caco-2 cells (a cell culture model of the intestinal mucosa commonly used in drug discovery), so it certainly will show good oral bioavailability in animals and humans." That conclusion may or may not be true. Without knowing that intestinal mucosal permeability is the critical factor that limits the oral bioavailability of this chemist's compound, it is incorrect to assume that this *in vitro* cell culture assay will predict oral bioavailability.

These examples clearly illustrate that companies have not only technical problems related to estimating oral bioavailability, but also "people" problems. The latter involve both educational gaps and communication issues. To resolve these problems, pharmaceutical companies, in cooperation with universities and professional associations, need to work together to educate scientists about how they can communicate with each other more effectively. Companies need to realize that, for all practical purposes, most scientists were formally educated as experts in narrowly focused fields and, most probably, experienced very little breadth in their formal university training. In addition, most scientists in the pharmaceutical industry have seldom been asked to communicate upstream or downstream from their location in the overall drug discovery and development process.

- How can professional associations help the pharmaceutical industry achieve the successful integration of drug discovery and drug development? As 2001 President of the American Association of Pharmaceutical Scientists (AAPS), I feel that associations such as AAPS can play a very important role in filling the educational gaps. AAPS, which has approximately 11,000 members, is an association that primarily serves scientists working in preclinical development in pharmaceutical companies and students who aspire to work in this part of the industry. Many AAPS members have significant scientific and managerial responsibilities for creating the technology to assess "drug-like characteristics" of molecules and in generating data that will be used to determine whether an NCE meets the company's "developability" criteria. One of my goals as AAPS President is to increase the participation of drug discovery and clinical discovery/development scientists in AAPS. I feel AAPS members are in a unique position to move beyond the characterization of NCEs to assist scientists in other disciplines to interpret these data and put them into the overall context of the "developability" of a compound. To accomplish this goal, I have asked the various sections and focus groups of AAPS to develop scientific programs that include topics in the boundary areas between drug discovery and drug development. AAPS will also work to foster strategic alliances with other scientific organizations that will lead to joint programming initiatives and the facilitation of information exchange. AAPS recently launched a new portal, AAPS Pharmaceutica, www.aapspharmaceutica.com, that will become a vital source of information on the technology used to characterize the "drug-like characteristics" of molecules and the interpretation of these data in light of the "developability" of NCEs. To enable ready access to this information, AAPS created a new membership service, the E-subscriber. This electronic-only membership service to AAPS provides the perfect conduit for scientists in other disciplines to obtain information being generated by AAPS members.

The rapid exchange of information between scientists in different disciplines will help to facilitate the overall integration of drug discovery and development in pharmaceutical companies. This will allow companies to reduce the attrition rates in preclinical and clinical development and help to insure the long-term growth and commercial success of research-based pharmaceutical companies in the United States.

Ronald T. Borchardt is President of the AAPS and Solon E. Summerfield Distinguished Professor, Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66047

Furth r Reading

1. S. Venkatesh and R.A. Lipper, "Role of the Development Scientist in Compound Lead Selection and Optimization", *Journal of Pharmaceutical Sciences*, 89(2):145-54, February 2000.

2. "Drug Discovery: Filtering Out Failures Early in the Game", *Chemical & Engineering News*, 78(23):63 June 5, 2000.

3. "*Pharma 2005: An Industrial Revolution in R&D*", Price Waterhouse Coopers: New York, 1998.

4. R.T. Borchardt, et al., (Eds), "*Integration of Pharmaceutical Discovery and Development: Case Histories*", Plenum Press: New York, 1998.

5. National Academy of Sciences, "*Graduate Education in the Chemical Sciences–Issues for the 21st Century*," National Academy Press: Washington, DC, 2000.

***The Scientist* 15[5]:43, Mar. 5, 2001**